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Oleifolioside A Mediates Caspase-Independent Human Cervical Carcinoma HeLa Cell Apoptosis Involving Nuclear Relocation of Mitochondrial Apoptogenic Factors AIF and EndoG

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ABSTRACT: Apoptosis, the main type of programmed cell death, plays an essential role in a variety of biological events. Whereas "classical" apoptosis is dependent on caspase activation, caspase-independent death is increasingly recognized as an alternative pathway. To develop new anticancer agents, oleifolioside A was isolated from *Dendropanax morbifera* Leveille and the biochemical mechanisms of oleifolioside A-induced apoptosis in HeLa cells were investigated. Exposure to oleifolioside A resulted in caspase activation and typical features of apoptosis, although cell death was not prevented by caspase inhibition. Oleifolioside A treatment induced up-regulation of Bad, loss of mitochondrial membrane potential, nuclear relocation of mitochondrial factors, apoptosis-inducing factor (AIF), endonuclease G (EndoG), and apoptosis induction. This is the first report of anticancer activity of oleifolioside A, and nuclear translocation of AIF and EndoG in oleifolioside A-treated HeLa cells might represent an alternative death signaling pathway in the absence of caspase activity.

KEYWORDS: *oleifolioside A, apoptosis, AIF, EndoG, HeLa*

INTRODUCTION

Apoptosis, or programmed cell death, is a highly regulated process that allows a cell to self-degrade in order for the body to eliminate unwanted or dysfunctional cells.[1] Apoptosis can be triggered in a cell through either the extrinsic pathway or the intrinsic pathway. The extrinsic pathway is initiated through the stimulation of transmembrane death receptors, such as Fas receptors, located on the cell membrane.[2] In contrast, the intrinsic pathway is initiated through the release of signal factors by mitochondria within the cell, and its two apoptotic pathways are executed mainly by a class of cysteine proteases known as caspases.[3] In addition, apoptosis-inducing factor (AIF) and endonuclease G (EndoG) can be released into the cytosol, which can be translocated to the nucleus and then cleave chromatin DNA independently of caspases.[4] Mitochondrial membrane dysfunction and the release of pro-apoptotic factors from the intermembrane space are controlled by molecules of the Bcl-2 members.[5] Pro-apoptotic molecules such as Bid and Bad serve as pore-forming pro-apoptotic molecules, whereas anti-apoptotic molecules such as Bcl-2 and Bcl-X_L prevent mitochondrial pore formation through binding with pro-apoptotic proteins of Bcl-2 members.[6]

Cervical carcinoma is one of the most common causes of death among women worldwide and threatens the health of women of reproductive age.[7] Cancer therapy is based on surgery, radiation therapy, and chemotherapy, which to date are not completely successful interventions. Thus, to improve clinical management of this disease, the development of new drugs is needed urgently. In recent years, natural compounds have gained considerable attention as chemotherapeutic agents because of their beneficial effects in overcoming tumor cell growth to apoptosis.[8−10]

Dendropanax morbifera Leveille (Araliaceae) has been used in traditional medicine for the treatment of several diseases, such as headache, infectious diseases, skin diseases, and malady.[11] It has been reported that the crude ethanol extract from the leaves of *Dendropanax arboreus* (Araliaceae) from Monteverde, Costa Rica, exhibits cytotoxic activity against Hep-G2, A-431, H-4IIE, and L-1210 tumor cell lines, but is not toxic against normal hepatocytes.[12] Recently, polyacetylene compounds have been isolated from *D. morbifera* and have been shown to have anticomplement,[13,14] antidiabetic,[15] and antiatherogenic activities in vitro.[16] Oleifolioside A, a cycloartane-type glycoside, was isolated from the lower stem of *Astragalus oleifolius* and has been shown to have antiplasmodial activity.[17] Our recent study

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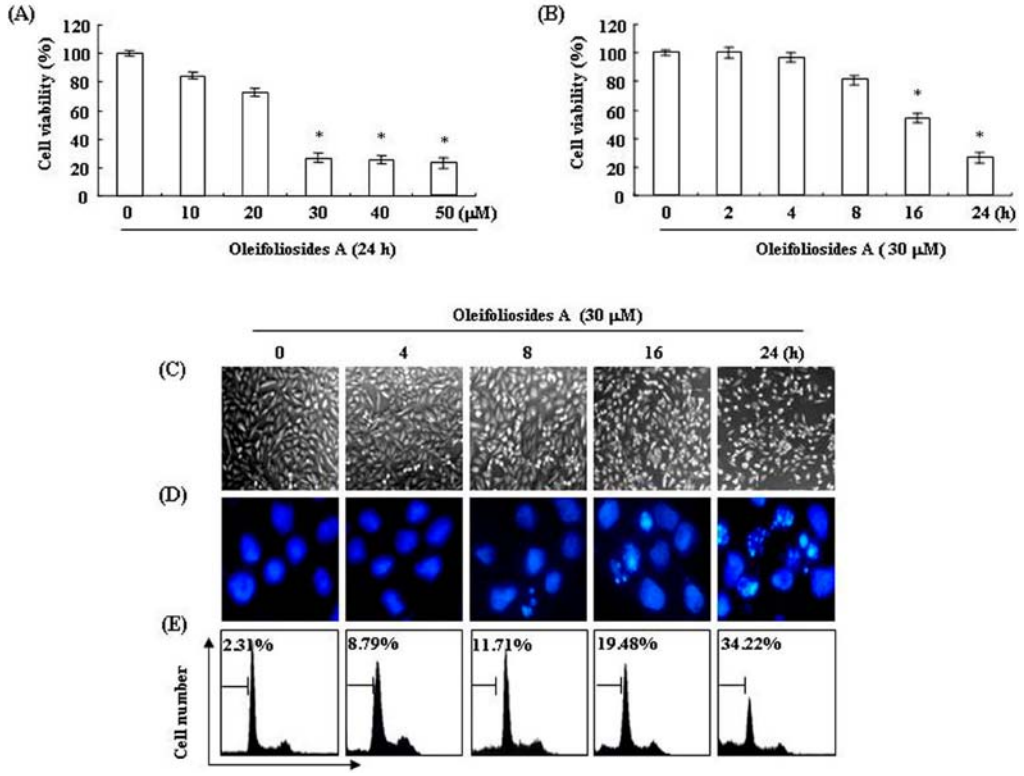

Figure 1. Effects of oleifolioside A on cell proliferation and apoptosis in HeLa cells. Cells were seeded at 5×10^4 cells/mL for 24 h and then treated with oleifolioside A for the indicated dose and time (A and B), and the cell viability was determined by MTT assay. Each point represents the mean ± SE of three independent experiments. The significance was determined using Student's *t* test (*, $p < 0.05$ vs untreated control). After treatment of the cells with 30 μM oleifolioside A for the indicated time, they were observed using an inverted microscope (upper panels, C, 200×) or were stained with DAPI solution for 10 min and then photographed with a fluorescence microscope using a blue filter (lower panels, D, 400×). Accumulation of sub-G1 population of cells under the same conditions as panel C and analyzed by flow cytometer (E).

evaluated the antidiabetic effects of dendropanoxide that has been extracted from *D. morbifera* in normal and streptozotocin-induced diabetic rats.[15] However, the signal molecular mechanisms of its anticancer activity have not been documented.

Recently, we isolated the bioactive agent, oleifolioside A from *D. morbifera*.[18] First, we use the oleifolioside A human cervical carcinoma HeLa cells as a model system with which to investigate the apoptotic mechanisms by which oleifolioside A induces cell death.

MATERIALS AND METHODS

Materials. 4,6-Diamidino-2-phenylindole (DAPI), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), doxorubicin, and propidium iodide (PI) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies specific for actin, AIF, Bcl-2, Bcl-X_L, Bax, Bad, Bid, cIAP-1, cIAP-2, caspase-3, caspase-8, caspase-9, cytochrome *c*, PARP, survivin, XIAP, goat anti-rabbit IgG-FITC, and goat anti-mouse IgG-FITC were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody specific for EndoG was purchased from Cell Signaling (Beverly, MA, USA). Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulin and an enhanced chemiluminescence (ECL) kit were purchased from Amersham (Arlington Heights, IL, USA). Z-VAD-fmk (a broad-spectrum caspase inhibitor) and 5,5′,6,6′- tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) were purchased from Calbiochem (San Diego, CA, USA). Oleifolioside A isolated from the lower stem parts of *D. morbifera* Leveille (Araliaceae) was prepared as described previously,[18] dissolved in dimethyl sulfoxide (DMSO) as a stock solution at 30 mM concentration, and stored in aliquots at −20 °C.

Cell Culture and Cell Viability Assay. The human cervical cancer HeLa cell line was obtained from the American Type Culture Collection and cultured in DMEM (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL). Cells were cultured at 37 °C in a humidified chamber containing 5% CO_2. The MTT reduction assay was used for determination of cell viability. In brief, HeLa cells (5×10^4 cells/mL) were seeded in 24-well plastic plates and treated with 30 μM oleifolioside A for 0−24 h and then treated with 0−50 μM oleifolioside A for 24 h, respectively. After various treatments, the medium was removed and the cells were incubated with 0.5 mg/mL of MTT solution. After incubation for 3 h, the supernatant was removed and formation of formazan was measured at 540 nm with a microplate reader.

Flow Cytometric Analysis. Following treatment for the indicated time with oleifolioside A, cells were collected, washed with cold PBS, and fixed in 75% ethanol at 4 °C for 30 min. Prior to analysis, the cells were washed once again with PBS. Flow cytometry analyses were performed using a DNA staining kit (CycleTEST PLUS Kit, Becton Dickinson, San Jose, CA, USA). The cell pellet was combined with 120 μL of solution A for 10 min at room temperature, after which 100 μL of solution B was added for 10 min at room temperature, and then 100 μL solution C was added for 30 min at 4 °C in the dark. The cells were then filtered through 35 mm mesh, and DNA content fluorescence was determined using a FACS Caliber (Becton Dickinson) flow cytometer within 1 h. The cellular DNA content was analyzed by CellQuest software (Becton Dickinson).

DAPI Staining. Cells were washed with ice-cold PBS and fixed with 4% paraformaldehyde (Sigma Chemical Co.) in ice-cold PBS for 10 min at room temperature. Fixed cells were stained with 2.5 μg/mL DAPI solution in PBS for 10 min at room temperature. Cells were

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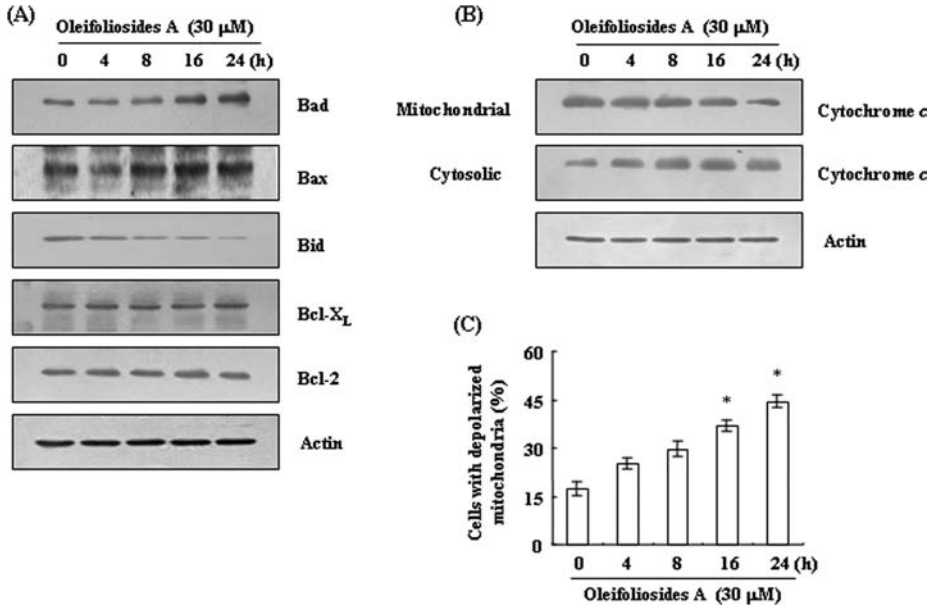

Figure 2. Effects of oleifolioside A on expression of Bcl-2 member proteins, loss of MMP ($\Delta\Psi$), and release of cytochrome *c* in HeLa cells. The cells were treated with 30 μM oleifolioside A for the indicated time; proteins on Western blots were detected with Bcl-2 family members (A) and cytochrome *c* (B) antibodies. Actin was used as an internal control. The cells were treated under the same conditions as in panel A, and loss of MMP (C) was measured by flow cytometer. Each point represents the mean ± SE of three independent experiments. The significance was determined using Student's *t* test (∗, $p < 0.05$ vs untreated control).

washed two more times with PBS and analyzed with a fluorescence microscope at 340−380 nm (Carl Zeiss, Germany).

Protein Extraction and Western Blot Analysis. Whole-cell protein extracts from HeLa cells were prepared with cell lysis buffer (20 mM sucrose, 1 mM EDTA, 20 μM Tris-HCl, pH 7.2, 1 mM DTT, 10 mM KCl, 1.5 mM $MgCl_2$, and 5 μg/mL aprotinin) for 30 min. Cells were disrupted by sonication and extracted at 4 °C for 30 min. The protein extracts were quantified using the Bio-Rad kit (Pierce). For Western blot analysis, lysate proteins (30−50 μg) were resolved over SDS−polyacrylamide gel electrophoresis and transferred onto nitrocellulose transfer membranes (Amersham). The membrane was blocked with 5% skim milk and incubated with the primary antibodies, followed by HRP-conjugated anti-mouse and anti-rabbit secondary antibodies. The detection of specific proteins was carried out with an ECL Western blotting kit according to the recommended procedure.[19]

Mitochondrial Membrane Potential (MMP, $\Delta\Psi_m$) Assay. The MMP of intact cells was measured by DNA flow cytometer with the lipophilic cation JC-1. JC-1 is a rationmetric, dual-emission fluorescent dye that is internalized and concentrated by respiring mitochondria and that can therefore reflect changes in MMP in living cells. There are two excitation wavelengths: at low values of MMP it remains a monomer (FL-1, green fluorescence; 527 nm), whereas it forms aggregates at high MMP (FL-2, red fluorescence; 590 nm) according to the recommended procedure (Calbiochem). For this study, the cells were trypsinized and the cell pellets were resuspended in PBS and incubated with 10 μM JC-1 for 20 min at 37 °C. The cells were subsequently washed once with cold PBS, suspended, and analyzed using flow cytometer.

Preparation of Mitochondrial Proteins. Cells were treated with oleifolioside A and harvested with ice-cold PBS. The mitochondrial and cytosolic fractions were isolated using a mitochondrial fractionation kit according to the manufacturer's instructions (Active-motif, Carlsbad, CA, USA). For Western blot analysis, an equal amount of protein (30 μg protein per lane) was subjected to SDS−polyacrylamide gels before transfer to the nitrocellulose membranes (Schleicher & Schuell) using standard protocols.

Immunocytochemistry. Cells were grown on coverslips and treated as indicated. Cells were washed twice in PBS, fixed with 4% paraformaldehyde in PBS at room temperature for 30 min, and then permabilized with 0.25% Triton X-100 solution for 10 min; the cells were subsequently incubated in a blocking solution of 1% bovine serum albumin (BSA) and incubated with primary antibodies for AIF and EndoG at room temperature for 1 h, respectively. After the incubation period, the study samples were rinsed four times with PBS and thereafter incubated with the secondary anti-rabbit-FITC and anti-mouse-FITC diluted 1:200 in buffer for 1 h at room temperature. Nuclei were stained with 2.5 μg/mL of DAPI and examined using a fluorescence microscope (Carl Zeiss, Germany).

Statistical Analysis. All data were derived from at least three independent experiments. Values were presented as the mean ± SE. Significant differences between the groups were determined using the one-way ANOVA. A p value of <0.05 was considered to be significant.

RESULTS

Oleifolioside A Induction of Apoptosis in HeLa Cells. To investigate the effects of apoptotic cell death induced by oleifolioside A, HeLa cells were treated with oleifolioside A at different dose levels and various amounts of time and subjected to an MTT assay. As shown in Figure 1A, following treatment with 0−50 μM oleifolioside A for 24 h, HeLa cells exhibited strong growth inhibitory effects. For a further assessment of oleifolioside A on the viability of HeLa cells, we examined the cell viability in treatment by varying the durations of treatment (0−24 h) and holding the concentration of oleifolioside A (30 μM) constant. This resulted in a significant reduction in cell viability in an oleifolioside A dose time-dependent manner (Figure 1B). In addition, direct observation using an inverted microscope revealed that numerous morphological changes occurred in cells treated with oleifolioside A. In particular, chromatin condensation, loss of nuclear construction, and formation of apoptosis bodies appeared in a time-dependent manner after oleifolioside A treatment (Figure 1C). To examine whether oleifolioside A inhibits the proliferation of HeLa cells by inducing apoptosis, cells treated with oleifolioside A were investigated after nuclear DAPI staining. The control cells exhibited the typical morphological features of apoptosis with nuclear fragmentation in the oleifolioside A treatment cells

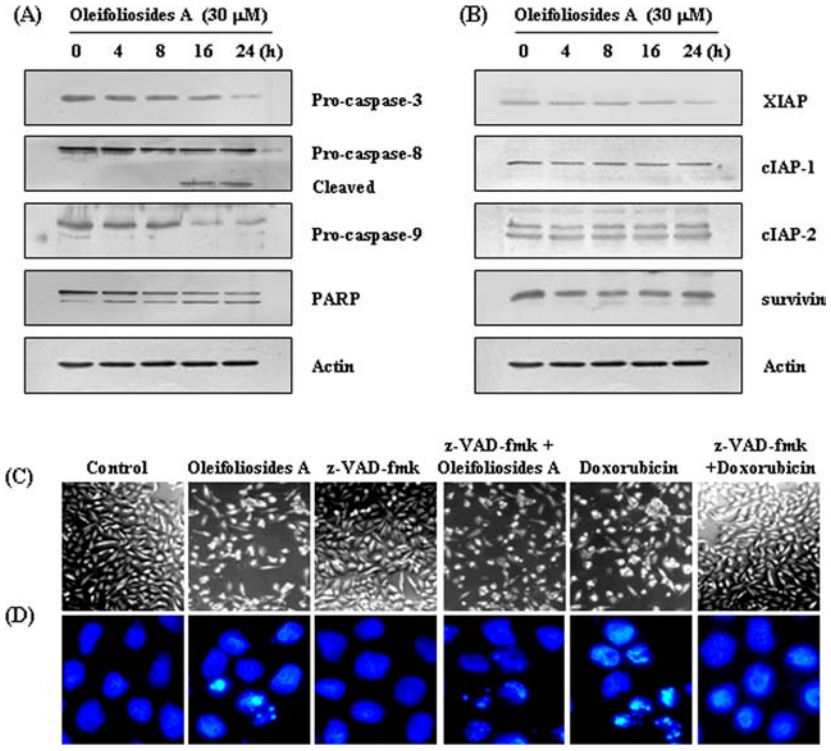

Figure 3. Effects of oleifolioside A on expression of IAP member proteins and caspase activation in HeLa cells. Cells were treated with 30 μM oleifolioside A for various amounts of time, and equal amounts of cell lysates (30 μg) were separated on SDS−polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were probed with the indicated antibodies (A, B). Actin was used as an internal control. The HeLa cells were pretreated 50 μM z-VAD-fmk for 1 h and then treated with 30 μM oleifolioside A or 2 μM doxorubicin for 24 h, and cell growth was observed using an inverted microscope (upper panels, C, 200×) or cells were stained with DAPI solution for 10 min and then photographed with a fluorescence microscope using a blue filter (lower panels, D, 400×).

(Figure 1D). Furthermore, flow cytometric analysis also revealed that treatment with oleifolioside A increased accumulation of cells at the apoptotic sub-G1 phase in a time-dependent manner (Figure 1E). These results indicate that the cytotoxic effects observed in response to oleifolioside A are associated with the induction of apoptotic cell death in HeLa cells.

Oleifolioside A-Induced Apoptosis Involves the Expression of Bcl-2 Family Members, Attenuation of MMP ($\Delta\Psi$), and Release of Cytochrome *c* in HeLa Cells. As shown in Figure 2A, an additional experiment was undertaken to elucidate whether oleifolioside A-induced apoptosis is involved in the expression of Bcl-2 family members. Cells were exposed to 30 μM oleifolioside A for 0−24 h, leading to an increase in expression of pro-apoptotic Bad and Bax proteins, and the level of total Bid protein was significantly decreased in response to oleifolioside A treatment in a time-dependent manner. However, the levels of anti-apoptotic Bcl-X_L and Bcl-2 remained unchanged. The role of the mitochondria in oleifolioside A-induced apoptosis of HeLa cells was further examined by investigating the effect of oleifolioside A on the levels of cytosolic and mitochondrial cytochrome *c*, as well as the MMP values. As shown in Figure 2B, exposure of cells to 30 μM oleifolioside A led to an increase in the release of the mitochondrial pro-apoptotic cytochrome *c* protein to the cytosol. In contrast, treatment with 30 μM oleifolioside A induced a decrease in cytochrome *c* protein in the mitochondria. Furthermore, we investigated the mitochondria in oleifolioside A-induced apoptosis of HeLa cells by measuring JC-1 dye retention. As shown in Figure 2C, exposing HeLa cells to oleifolioside A significantly reduced mitochondria dysfunction in a time-dependent manner. These results suggest a direct role of the mitochondrial in oleifolioside A-induced apoptosis in HeLa cells.

Oleifolioside A Induces Apoptosis through Caspase-Independent HeLa Cells. Next, we investigated whether oleifolioside A-induced apoptosis is involved in the activation of caspases and the subsequent proteolytic cleavage of poly(ADP-ribose) polymerases (PARP) in HeLa cells. As shown in Figure 3A, Western blot analysis suggested that treatment with 30 μM oleifolioside A led to a significant decrease in the procaspase-3, procaspase-9, cleavage of caspase-8, and PARP proteins. Furthermore, IAP member proteins were determined in oleifolioside A-induced apoptosis by Western blot analysis. As shown in Figure 3B, treatment with oleifolioside A did not change the expression levels of IAP member proteins. To further investigate the significance of caspase activation in oleifolioside A-induced apoptosis, HeLa cells were pretreated with z-VAD-fmk (50 μM) for 1 h, followed by treatment with 30 μM oleifolioside A or 2 μM doxorubicin for 24 h. Pretreatment with z-VAD-fmk attenuated doxorubicin-induced chromatin condensation and formation of apoptotic bodies. Interestingly, pretreatment with z-VAD-fmk failed to suppress the oleifolioside A-induced morphological changes, chromatin condensation, and appearance of apoptotic bodies (Figure 3C,D). Taken together, these results suggest that oleifolioside A-induced apoptosis is independent of caspase activation in HeLa cells.

Oleifolioside A Triggers the Release of AIF and EndoG from Mitochondria. To investigate the caspase-independent

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apoptosis factors of oleifolioside A-induced HeLa cells, we examined the expression levels of mitochondrial factors AIF and EndoG, which are considered to induce caspase-independent apoptosis. As shown in Figure 4, Western blot

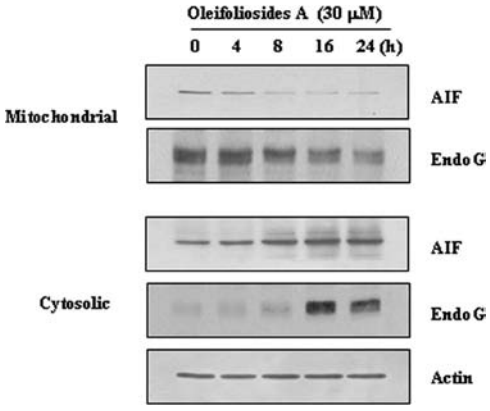

Figure 4. Effects of oleifolioside A on AIF and EndoG from the mitochondria to cytoplasm in HeLa cells. Cells were treated with 30 μM oleifoliosides A for various amounts of time. Equal amounts of cytosolic and mitochondrial proteins were extracted, and proteins on Western blots were detected with the indicated antibodies and ECL.

analysis showed that 30 μM oleifolioside A led to a significant release from the mitochondrial to cytosolic levels of AIF and EndoG., which occurred in a time-dependent manner. Furthermore, the cellular localization of AIF and EndoG by the oleifolioside A treatment was determined in HeLa cells. Immunofluorescence studies confirmed translocation of AIF and EndoG to the nucleus after treatment with 30 μM oleifolioside A for 24 h (Figure 5). Taken together, these results suggest that oleifolioside A induces apoptosis through caspase-independent and -dependent nuclear relocation of mitochondrial apoptogenic factors AIF and EndoG.

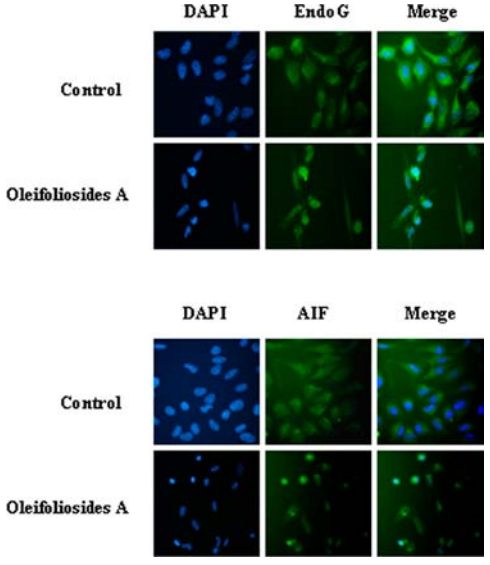

Figure 5. Effects of oleifolioside A on nuclear relocation of mitochondrial AIF and EndoG in HeLa cells. Cells were incubated with 30 μM oleifolioside A for 24 h; fixed cells were stained with DAPI and anti-AIF, anti-EndoG antibody, and then FITC-conjugated anti-mouse IgG or anti-rabbit IgG. Images were taken by a fluorescence microscopy.

DISCUSSION

The present study showed that after exposure to the potent antiplasmodial new bioactive agent of oleifolioside A,[18] HeLa cells displayed typical apoptotic appearances (i.e., nuclei condensation, cell shrinkage, and apoptotic bodies) along with accumulation of a sub-G1 cell cycle population, pointing to an intrinsic apoptotic pathway in HeLa cells. Although the caspase activation occurred in oleifolioside A-treated HeLa cells, a broad-spectrum caspase inhibitor, z-VAD-fmk, failed to increase the cell survival, indicating that the uncoupling of the caspase cascade to oleifolioside A induced HeLa cell apoptosis. Examination of the apoptotic effector proteins (AIF and EndoG), targeted by increasing of pro-apoptotic Bcl-2 member proteins, appeared to show that nuclear translocation of AIF and EndoG could be caused by a loss of MMP in HeLa cells.

Mitochondria play a major role in the apoptotic process, through the release of cytochrome *c* and other pro-apoptotic proteins that normally reside in the intermembrane space between the inner and outer mitochondrial membranes.[5,20] It is known that the release of cytochrome *c* from mitochondria is an important process during apoptosis induced by chemotherapeutic agents, and several pro-apoptotic and anti-apoptotic proteins are involved upstream and downstream of mitochondria.[21−23] Cytochrome *c* is a pro-apoptotic regulator of mitochondrial-dependent apoptosis by several different stimuli.[24−26] In death ligand-induced apoptosis, activation of caspase-8 cleaves a cytosolic substrate Bid, leading to release of cytochrome *c*. In this study, the pro-apoptotic Bax and Bad but not death receptors such as DR4, DR5, Fas, and FasL (data not shown) increased release of cytochrome *c* from mitochondria by oleifolioside A-induced apoptosis. These results suggest that oleifolioside A-induced apoptosis was via an intrinsic pathway. Some papers propose that when p53 accumulates in the cytosol, it can function analogously to the BH3-only subset of pro-apoptotic Bcl-2 proteins to activate Bax or requires reactive oxygen species (ROS) to trigger apoptosis in HeLa cells.[27,28] However, the regulation of the p53 or ROS level was not examined in oleifolioside A-treated HeLa cells. Furthermore, apoptotic effects by oleifolioside A were not inhibited by treatment with *N*-acetylcysteine (data not shown), suggesting that the release of cytochrome *c* and apoptosis induced by oleifolioside A do not require regulation of p53 and generation of ROS.

Caspases belong to a family of cysteine proteases that are integral components of the apoptotic pathway.[29] Recently, many studies have determined that a variety of chemotherapeutic agents induce apoptosis or enhance apoptosis through the activation of caspases.[19,30,31] However, Iwasaki et al. have reported that chemopreventive agents cause caspase-independent necrosis-like cell death in chronic myelogenous leukemia.[32] Lee et al. also reported epigallocatechin-3-gallate (EGCG), a major green tea polyphenol caspase, induces caspase-independent apoptotic cell death via mitochondria with the release of AIF and EndoG in Hep2 cells.[33] It is unclear as to whether caspase activation was involved in cell death of cervical cancer by chemopreventive agents. In the present study, oleifolioside A induces activation of caspase-3, -8, and -9, but cell death was not inhibited by treatment with z-VAD-fmk. These results indicate that oleifolioside A-induced apoptosis occurred via a caspase-independent pathway. Some previous papers have shown that human X-chromosome-linked IAP directly inhibits at least two members of the caspase family of

cell-death proteases, caspase-3 and caspase-7.[34] Inhibitors of apoptosis proteins (IAPs), which include cIAP1, cIAP2, survivin, and X chromosome-encoded IAP (XIAP), regulate apoptosis in a negative manner through inhibition of caspases.[35] Greer et al. have shown chemotherapies that induce cell death through the mitochondrial pathway required only inhibition of IAP member proteins for sensitization of non-small-cell lung cancers.[36] Liu et al. reported rapid induction of mitochondrial events and caspase-independent apoptosis in survivin-targeted melanoma cells,[37] suggesting that IAP member proteins may play a crucial role in oleifolioside A-induced apoptosis. However, in the present study, regulation of the IAPs was not examined in oleifolioside A-treated HeLa cells. These results suggested that oleifolioside A-induced apoptosis in HeLa cells was caused by other apoptotic triggers via a caspase-independent pathway.

The mitochondria have previously been shown to play a crucial role in many apoptotic responses through the release of mitochondrial apoptogenic proteins, such as AIF and EndoG, which is independent of the caspase cascades.[4] AIF is known to contribute to caspase-independent cell death, whereby its release from the mitochondria to the nucleus causes chromatin condensation and cell death.[38−40] Because the case of HeLa cell death shown here is caspase-independent, we have suspected the contribution of AIF to the oleifolioside A-induced cell death. This suggestion is supported by the findings that increased nuclear levels of AIF were observed in oleifolioside A-treated HeLa cells. In addition, we have found an increase in nuclear levels of another mitochondrial factor, EndoG, which also triggered caspase-independent cell death.[41−43] Significant AIF and EndoG were detected in the cytosol, and their translocated protein was observed in the nucleus during oleifolioside A-mediated apoptosis in HeLa cells, which indicates that AIF and EndoG might act as terminal key molecules. Some previous papers have shown that cell death was induced by Bax-dependent mitochondrial permeability without caspase activity and requires caspase activation by processes involving AIF/EndoG-dependent pathways.[44,45] This is consistent with the present study showing that an increase of the Bax and Bad protein levels by oleifolioside A treatment may promote the release of AIF and EndoG from the mitochondria. It must be noted, however, that the regulation of the AIF and EndoG levels by Bax and Bad was not examined in oleifolioside A-treated HeLa cells.

In summary, oleifolioside A induced apoptosis through a caspase-independent pathway by the up-regulation of the pro-apoptotic Bcl-2 family, resulting in a loss of mitochondrial membrane potential and the mitochondrial release of cytochrome *c*, followed by the translocation of AIF and EndoG from the cytoplasm into the nucleus of HeLa cells.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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